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Research Article

ANTIBIOTIC SENSITIVITY PATTERN IN NEONATAL INTENSIVE CARE UNIT OF A TERTIARY CARE HOSPITAL OF INDIA

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ABSTRACT

Objective:To do audit of the antimicrobial (AM) sensitivity pattern in Neonatal Intensive Care Unit (NICU).
Methodology:This retrospective case series based on a case record form analysis of a tertiary care hospital of NICU was carried out for the duration Jan-2010 to Mar-2011. Data of demographics, diagnosis, type of infection, indication of the use of the AMs, hospital stay, specimen, causative agent, sensitivity pattern, prescribed antibiotics were collected in a case record form. Antibiotic sensitivity testing was performed through CLSI method.
Result:Out of 676 admitted neonates, 52 neonates showed bacteriological proven infection in 58 cultures. Septicemia – 88.46% (56.52% early onset and 43.48% late onset) was the predominant cause of infection. *S. epidermidis* (37.93%) was the most commonly isolated organism followed by *K. pneumoniae* (22.41%). 78.78% and 44% of isolated strain of gram positive and gram negative have shown multiple resistance (p=0.012). Gentamicin (100%), vancomycin (96.87%), and ciprofloxacin (90.9%) were most sensitive drugs for GPIs. 68.19% and 55.56% of isolated strains of *S. epidermidis* and *S. aureus* were resistant to cloxacillin. Levofloxacin (100%), imipenem (100%), meropenem (100%), ofloxacin (88%) and gentamicin (85.71%) were most effective drugs for GNIs. Sensitivity of third generation cephalosporins for GNIs were: cefotaxime (57.14%), ceftriaxone (57.14%), cefoperazone (50%) and ceftazidime (50%). The most commonly used AMs were cefotaxime (59.09%), amikacin (55.45%), levofloxacin (54.55%) and piperacillin + tazobactam (51.82).
Conclusion:Cloxacillin resistant gram positive organisms and third generation cephalosporins resistant gram negative bacilli were the predominant antimicrobial resistant organisms.

Keywords: antibiogram, antibiotic sensitivity, bacterial resistance, neonatal intensive care unit.

INTRODUCTION

In Neonatal Intensive Care Unit (NICU), neonatal sepsis is the most common infection to occur and most of studies for NICU are targeted to it[1-3]. Septicemia is the major cause of neonatal mortality and morbidity worldwide[2]. It can be early or late onset septicemia. In suspected septicemia, two or three days empirical antibiotic therapy should begin immediately after cultures have been obtained. Antibiotics should be re-evaluated when the results of the cultures and sensitivity are available[4]. Multidrug antibiotic resistance is an emerging problem in NICU particularly in developing countries. Moreover, the spectrum of organisms that cause neonatal sepsis changes from time to time and varies from region to region. Continuous surveillance for antibiotic susceptibility, rational use of antibiotics and the strategy of antibiotic cycling can provide some answers to it[5,6]. Timely review of sensitivity pattern locally in each hospital can guide about the development of resistance to first line and higher class of AMs. It helps the clinicians in selecting AMs rationally. Currently, there is no accepted national database of antimicrobial resistance for different pathogens in India except for those where there is a specific national health programme[7]. This is a concerned issue now-a-days.

MATERIALS AND METHODS

This retrospective, observational tertiary care hospital based study was carried out after the approval from the Institutional Review Board (IRB), Government Medical College, Bhavnagar, Gujarat, India. Consent waiver was obtained for the evaluation of the data. Indoor case papers, prescription papers and AM sensitivity reports of NICU from Jan-2010 to March-2011 (15 months) were collected from the case record section of Sir T. General Hospital, Bhavnagar. Information was collected for the demographic data of the patient, admission unit, duration of hospital stay, diagnosis, type of infection (nosocomial or primary), empirical treatment, indication of the use of the AMs, collected specimen, causative agent, sensitivity pattern, treatment after sensitivity pattern in case record form.

Standard procedures of culture and sensitivity were followed. Initially the specimens were studied for identification of isolates by gram stains and culture growth on nutrient, blood and MacConkey agar. Colonies from nutrient agar were used for biochemical tests and antibiotic sensitivity. After confirmation of the organism, culture growth was tested for *in vitro* antibiotic susceptibility testing performed by disc diffusion method (modified Kirby Bauer method) on Muller Hinton agar. Quality control for gram stains, biochemical tests, culture media and disc diffusion methods were maintained daily as per Clinical and Laboratory Standard Institute (CLSI) guidelines. External quality assurance scheme was also maintained periodically[8].

Statistical Analysis

Descriptive statistics were used for analysis. Data were expressed as proportions for the isolated organisms, infections, isolated organisms for particular infection and sensitivity patterns, etc. Resistance to two or more drugs was considered multiple resistances. Percentage of multiple resistances was calculated for isolated strain. Chi-square test was used to compare the multiple resistances between gram positive and negative organisms. All the statistical analysis was done through Graph Pad prism software demo version. $P<0.05$ was considered statistically significant.

RESULTS

Total 676 neonates admitted in NICU over a period of 15 months. 126 cultures were sent from 110 clinically suspected cases (70 male and 40 female). Specimens used were: blood (104), CSF (10), umbilical tip (3), central vein line tip (2), swab (2), catheter tip (1), endotracheal tube (1), pus (1), scalp abscess (1) and urine (1). Organisms were isolated in 58 cultures from 52 neonates (35 male and 17 female) which gives bacteriological proven infection rate of 7.69%. Out of 110 cases, 89 were discharged (41 had positive culture during admission). 6 were transferred to a higher centre (1 had positive culture during admission), and 15 died (10 had positive cultures during admission). In 58 positive cultures, 33 gram positive and 25 gram negative organisms were isolated. 26 (56.52%) neonates developed early onset and 20 (43.48%) developed late onset septicemia. Out of 10 deaths in bacteriological proven infection cases, from 9 neonates gram positive organism (8 cases, *S. epidermidis*) were isolated.

Isolation pattern of organisms and infection patterns are given in Table 1. The most common isolated organisms were *Staphylococcus epidermidis* (37.93%) and *klebsiella pneumoniae* (22.41%). Most common infection was septicemia (88.46%). The most common organisms causing septicemia were *S. epidermidis* (32.76%) and *K. pneumoniae* (18.97%). *S. epidermidis* was isolated in 34.61% and 45% cases of early and late onset septicemia respectively. 67.79% of

isolated strains have shown multiple resistances. Most of the isolated strains of *S. epidermidis* and *S. aureus* have shown multiple resistances. Overall, 78.78% and 44% of isolated strain of gram positive and gram negative has shown multiple resistances (*P*=0.012).

Sensitivity pattern of AMs for gram positive isolates (GPIs) are presented in Table 2. Most sensitive AMs against *S. epidermidis* were vancomycin (100%), gentamicin (100%), clindamycin (95.23%) and ciprofloxacin (90.90%). Most sensitive AMs against *S. aureus* were ciprofloxacin (100%), gentamicin (100%), amikacin (100%), cefotaxime (100%) and vancomycin (90%). Higher sensitivity of vancomycin, clindamycin, tetracycline and macrolides was observed for *S. epidermidis* than *S. aureus.* Ciprofloxacin, cefotaxime, amikacin were more sensitive AMs for *S. aureus* than *S. epidermidis.* Both gram positive organisms have shown reduced sensitivity for cloxacillin.

Sensitivity pattern of AMs for gram negative isolates (GNIs) are presented in Table 3. *K. pneumonia*, *E. coli*, *A. baumannii* and *P. aeruginosa* were 100% sensitive to levofloxacin, imipenem and meropenem. Other sensitive AMs for *K. pneumonia* were ofloxacin (92.3) and gentamicin (87.5).

Table 1: Percentage of infections along with isolation pattern of different organisms with multiple resistances in NICU. (Data are expressed in absolute number of the organisms and values in parenthesis are in percentage)

Isolated organisms	Septicemia No. (%)	Urinary Tract Infections No. (%)	Meningitis No. (%)	Respira-tory Infections No. (%)	Umbilical cord Infection No. (%)	Total No. (%)	Multiple resistan-ces
S. epidermidis	19 (32.76)	1 (1.72)	-	1 (1.72)	1 (1.72)	22 (37.92)	18 (81.81)
K. pneumoniae	12 (20.69)	-	-	1 (1.72)	1 (1.72)	14 (24.13)	7 (50)
S. aureus	10 (17.24)	-	-			10 (17.24)	7 (70)
E. Coli	4 (6.9)	-	-			4 (6.9)	1 (25)
A. baumannii	3 (5.17)	-	1 (1.72)			4 (6.9)	2 (50)
P. aeruginosa	3 (5.`17)	-	-			3 (5.17)	1 (33.33)
β hemolytic streptococci	1 (1.72)	-	-			1 (1.72)	1 (100)
Total	52 (89.65)	1 (1.72)	1 (1.72)	2 (3.44)	2 (3.44)	58 (100)	37 (63.79)

Table 2: Antibiogram of GPIs in NICU.

Antimicrobial Agent	Sensitivity in percentage (sensitive isolate/tested isolate)	
	Staphylococcus aureus	***Staphylococcus epidermidis***
Penicillin	0 (0/7)	11.11 (1/9)
Cloxacillin	44.44 (4/9)	31.81 (7/22)
Vancomycin	90 (9/10)	100 (21/21)
Cotrimoxazole	60 (6/10)	40.9 (9/22)
Clindamycin	80 (8/10)	95.23 (20/21)
Ciprofloxacin	100 (10/10)	90.9 (20/22)
Levofloxacin	NT	66.66 (2/3)
Gentamicin	100 (6/6)	100 (8/8)
Amikacin	100 (7/7)	75 (6/8)
Tetracycline	66.66 (2/3)	85.71 (12/14)
Erythromycin	30 (3/10)	45.45 (10/22)
Roxithromycin	28.57 (2/7)	55.55 (5/9)
Clarithromycin	28.57 (2/7)	71.42 (5/7)
Azithromycin	42.85 (3/7)	75 (6/8)
Cefotaxime	100 (7/7)	87.5 (7/8)
Cefoperazone	100 (7/7)	100 (8/8)

Table 3: Antibiogram of GNIs in NICU.

Antimicrobial agent	Sensitivity in percentage (sensitive isolate/tested isolate)			
	E. Coli	***K. pneumoniae***	***P. aeruginosa***	***A. baumannii***
Ciprofloxacin	60 (3/5)	76.92 (10/13)	100 (3/3)	50 (2/4)
Ofloxacin	100 (5/5)	92.3 (12/13)	66 (2/3)	75 (3/4)
Levofloxacin	100 (5/5)	100 (13/13)	100 (3/3)	100 (4/4)
Gentamicin	75 (3/4)	87.5 (7/8)	100 (1/1)	100 (1/1)
Netilmicin	75 (3/4)	75 (6/8)	100 (1/1)	0 (0/1)
Amikacin	75 (3/4)	50 (4/8)	100 (1/1)	100 (1/1)
Tobramycin	60 (3/5)	69.23 (9/13)	100 (3/3)	75 (3/4)
Imipenem	100 (5/5)	100 (13/13)	100 (3/3)	100 (4/4)
Meropenem	100 (5/5)	100 (13/13)	100 (3/3)	100 (4/4)
Ceftriaxone	75 (3/4)	50 (4/8)	100 (1/1)	0 (0/1)
Ceftazidime	50 (2/4)	37.5 (3/8)	100 (1/1)	100 (1/1)
Cefoperazone	75 (3/4)	37.5 (3/8)	100 (1/1)	0 (0/1)
Cefotaxime	75 (3/4)	50 (4/8)	100 (1/1)	0 (0/1)
Ampicillin + Sulbactam	25 (1/4)	16.66 (1/6)	66.66 (2/3)	100 (1/1)
Piperacillin	NT	NT	50 (1/2)	NT
Piperacillin + Tazobactam	NT	NT	100 (2/2)	NT
Colistin	NT	NT	50 (1/2)	NT

NT = Not Tested

As shown in Fig 1, gentamicin (100%), vancomycin (96.87%), ciprofloxacin (90.90%), clindamycin (90.62%) and amikacin (86.66%) have shown good spectrum activity against GPIs. Levofloxacin (100%), imipenem (100%), meropenem (100%),

ofloxacin (88%) and gentamicin (85.71%) have shown good spectrum activity against GNIs [Fig 2].

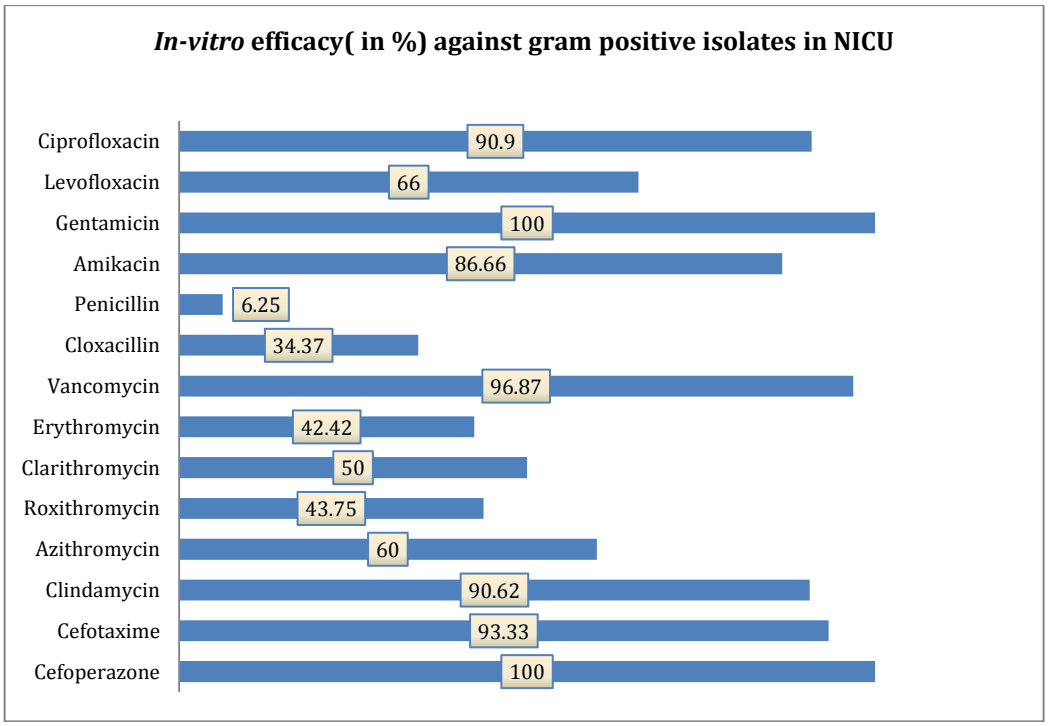

Fig 1: It shows *In-vitro* efficacy of different AM agents against GPIs in NICU.

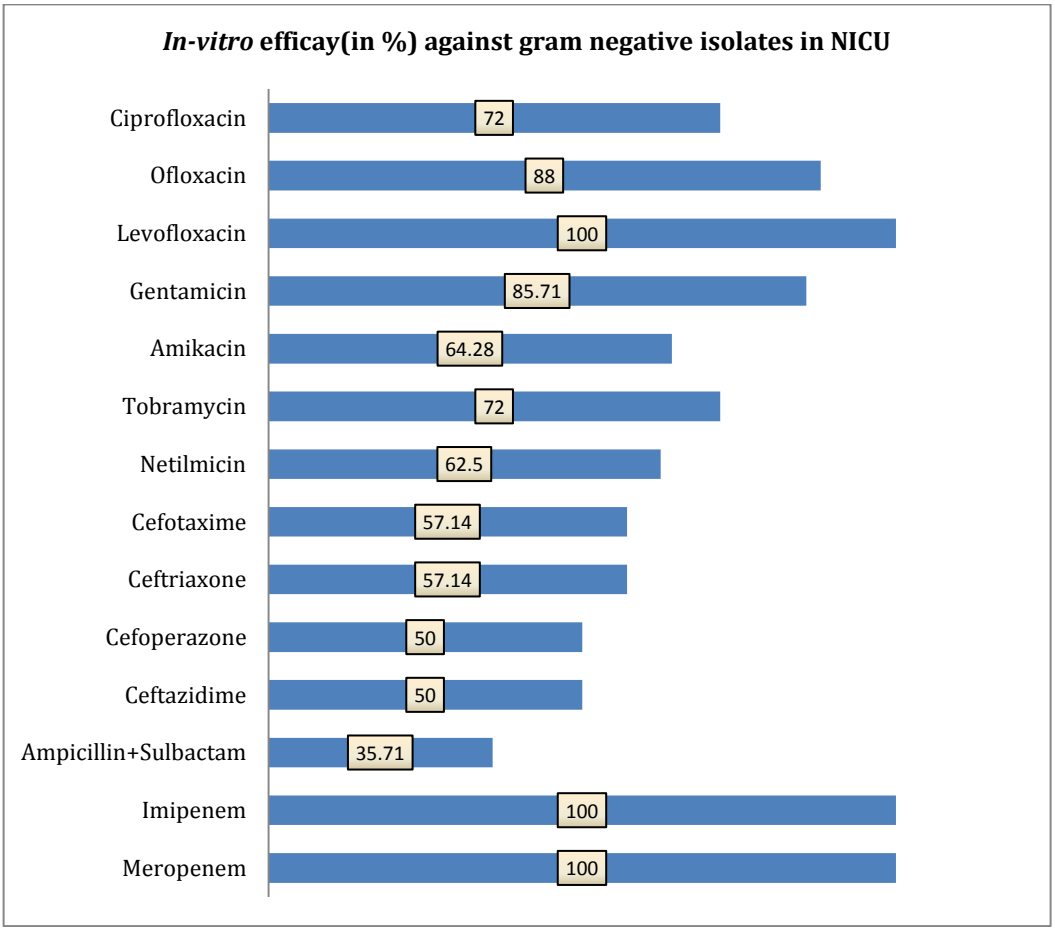

Fig 2: It shows *In-vitro* efficacy of different AM agents against GNIs

Median prescription of AMs per neonates was 2 (Min-0, Max-8). Overall AM consumption is presented in Table 4. The most commonly used drugs were cefotaxime (59.09%), amikacin (55.45%), levofloxacin (54.55%) and piperacillin + tazobactam (51.82%). The most commonly used combinations were cefotaxime + amikacin (55.45%) followed by levofloxacin + piptaz (piperacillin

+ tazobactam) (51.82%), ampicillin + gentamicin (14.55%) and meropenem + teicoplanin (10.91%).

Table 4: Prescription pattern of AMs in NICU.

Antimicrobial agent	Percentage of patient receiving AM agent
Cefotaxime	59.09
Amikacin	55.45
Levofloxacin	54.55
Piperacillin + Tazobactam	51.82
Gentamicin	14.55
Ampicillin	14.55
Teicoplanin	10.91
Meropenem	10.91
Metronidazole	8.18
Crystalline Penicillin	4.55
Vancomycin	3.64
Amoxicillin + Clavulanic acid	3.64
Cefoperazone + Sulbactam	3.64
Netilmicin	2.73
Ciprofloxacin	1.82

DISCUSSION

Neonates are more susceptible to sepsis and nosocomial infections. The emergence of AM resistance in NICU affects the management of it. It affects the initial choice of empirical AMs and also leads to multiple AM prescription. Multiple AMs increase the chances of drug interactions, adverse drug reactions, and reduces the future choice of AMs by promoting the resistance. It also prolongs the intensive care stay and increase the cost of treatment. Auditing of AM sensitivity pattern guides about the development of early resistance and helps in selecting appropriate AMs.

Organisms were isolated in 58 (46.03%) out of 126 cultures. Blood (104) being the most commonly used specimen showed 49 (47.11%) positive cultures compared to 13.17 to 54% of other studies[1,2,9]. Septicemia (88.46%) was the most common infection in our study. Gram positive (58.70%) septicemia was more common in our NICU as against gram negative septicemia in the other studies (58% to 80.40%)[1,10,11], *S. epidermidis* (37.93%) was predominant pathogens for septicemia in our study as compared to *K. pneumonia* (28.3%)[2], *E.coli* (37%)[1,12], *S. aureus* (29.5% and 42.75%)[9,13]. In our study isolation rate of *K. pneumonia, S. aureus, E. coli and A. baumanii* were 22.41%, 18.37%, 6.12% and 6.12% respectively. Organisms most frequently isolated in the NICU are likely to be vary with time to time and hospital settings. More than 50% of isolated strains of *S. epidermidis, S. aureus, β hemolytic streptococci* and *K. pneumonia* have shown multiple resistances. Multiple resistances (resistance to two or more drugs) were observed in 78.78% and 44% of gram positive and gram negative bacteria respectively. Observation of previous study was 45.7% and 84.2% for gram positive and gram negative bacteria respectively[3]. Mortality was also high in gram positive (particularly *S.* epidermidis) isolated neonates in our study.

Regarding the GPIs, both *S. aureus* and *S. epidermidis* have shown almost complete resistance to penicillin. Cloxacillin resistance to *S. aureus* and *S. epidermidis* exceeds 50% as reported in other tertiary care centers of India, Nepal and Saudi Arabia[13,14,15]. Cloxacillin resistance highly prevalent in our setup. Resistance of staphylococci to methicillin is mediated by a specific penicillin binding protein that has a reduced affinity for beta-lactam antibiotics. It is consequently to all beta-lactams upon acquisition of the *mecA* gene[16]. It circulate within hospital settings and can act as a reservoir of mobile genetic elements transferred to other *S. epidermidis* isolates as well *S. aureus*[15,16]. We found one Vancomycin resistant *S. aureus* (VRSA) over the duration of 15 months while one Indian study did not found any VRSA over the duration of four years[2]. However, cross checking of VRSA with other laboratory was not done. Vancomycin resistance has been very low in tertiary care centers of India as per one article[13]. Coagulase-negative staphylococci have acquired multiple resistances[17]. Third generation cephalosporins have shown good sensitivity for GPIs. We have observed reduced sensitivity of *S. aureus* to macrolides. Erythromycin resistance in our study was 30 % against 46.5% in another study[2]. Azithromycin sensitivity in our setup was 60% against 100% in one Nigerian study[9]. We observed higher sensitivity of aminoglycosides particularly, gentamicin for gram positive organisms. Sensitivity of *S. aureus* was 100% for gentamicin against 50% to 62.5% in other studies[2,9]. Sensitivity of ciprofloxacin is 100% for *S. aureus* and 90.90% for *S. epidermidis* in our study. Another setup noted 85% sensitivity of ciprofloxacin for *S. aureus*[1].

Regarding the GNIs, all have shown the complete sensitivity to carbapenems. Among fluroquinolones levofloxacin has shown highest sensitivity for gram negative organisms in comparison to ofloxacin and ciprofloxacin in spite of its widespread use. Our study has shown higher resistance of *K. pneumoniae* and *E. coli* for third generation cephalosporins. Overall sensitivity pattern of GNIs as compared to other study is: cefotaxime (57.14% vs. 14% to 66%), ceftriaxone (50% vs. 86.4%), and ceftazidime (23.53% vs. 28.4% to 60%)[2,9,10]. This may be because of widespread use of cefotaxime. As a first line empirical treatment 59% of patients were receiving cefotaxime in NICU. Its use should be restricted to reduce the development of the further resistance. Previous studies from India have reported ESBL (extended spectrum ß lactamase) production among GNIs (varying from 18%-86%) from neonatal septicemia patients[17]. From aminoglycosides, amikacin has reported reduced sensitivity as compared to gentamicin against most commonly isolated organism *K. Pneumonia* which suggests lack of cross resistance between aminoglycosides. This may be due to higher use of amikacin (55.45%) as first line empirical treatment over gentamicin (14.55%) in our setup. Increased pressure through use of amikacin and cefotaxime in the NICU might have selected for resistant strains. Gram-negative bacteria showed high-level resistance to ampicillin, similar to previous studies[3,10,12].

AM combinations used in our NICU were: cefotaxime + amikacin (55.45%), levofloxacin + piptaz (piperacillin + tazobactam) (51.82%), ampicillin + gentamicin (14.55%) and meropenem + teicoplanin (10.91%). Considering the development of resistance to cefotaxime to gram negative organisms its use should be restricted. Amikacin can be replaced by gentamicin. Use of ampicillin should also be restricted. Carbapenems and vancomycin should be used as a second line drug.

CONCLUSION

Septicemia was the predominant cause of infection in neonates. *S. epidermidis* was the predominant isolated organism in both early and late onset septicemia. Cloxacillin resistant gram positive organisms and third generation cephalosporins resistant gram negative bacilli were the predominant antimicrobial resistant organisms. Multiple resistance and mortality were high in gram positive isolated neonates. Vancomycin, fluroquinolones, aminoglycosides and third generation cephalosporins have shown good sensitivity for gram positive organism. carbapenems, levofloxacin and gentamicin have shown good sensitivity for gram negative isolates.

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